protofilaments. To address this point, we examined dynein motility using zinc-induced tubulin sheet (Zn-sheet) which has an arrangement of adjacent protofilaments with anti-parallel and opposite orientations. From the structural analysis, the dynein binding sites are revealed to be exposed only at either edge of a Zn-sheet. Previously, we have shown that the Zn-sheet move on the glass surface coated with the dynein or kinesin molecules. Unlike microtubules, Zn-sheets followed the winding path and the tracks are often circular. In this study, we aimed to observe the processive movement of single dynein molecules on the edge of a Zn-sheet by TIRF microscopy. As a result of deliberate preparations, we have successfully observed that single dynein molecules walk on Zn-sheets. The velocity of the movement on Zn-sheets was almost the same as that on microtubules. These results demonstrate that the single protofilament is enough to support dynein motility, and suggest that dynein uses only one protofilament of microtubules as well as one protofilament at the edge of Zn-sheets. Considering the large size of the dynein head, it is hard for the two heads of the dynein molecules to move on a single protofilament by the hand-over-hand mechanism advocated for the two heads of kinesin, and it is necessary to investigate the coordination of the two head of dynein.

2477-Pos Board B463

Two-Dimensional Analysis of the Dynein Stepping Mechanism

Weihong Qiu, Nathan Derr, Andrew P. Carter, Samarea L. Reck-Peterson.

Cytoplasmic dynein powers the transport of diverse macromolecules within eukaryotic cells, enabling them to effectively organize their contents, move, divide and respond to signals. One of our goals is to understand how dynein moves processively along microtubules to perform these functions. Our initial analysis of recombinant *S. cerevisiae* cytoplasmic dynein stepping was a one-dimensional analysis, in which all movements were projected along the axis of the microtubule (Reck-Peterson et al., 2006). However, as we also observed dynein side-stepping in this earlier study, we have now performed a complete analysis of dynein stepping in two-dimensions. Our analysis uses a new two-dimensional step finding algorithm to determine dynein's true step size, stepping angle and frequency of off-axis stepping. In these experiments we have used high-precision single molecule fluorescence microscopy to analyze the stepping behavior of dynein labeled with a single quantum dot on its tail domain. When we analyze dynein's step size using on-axis projection we detect a predominant step size of 8nm, consistent with our previous studies. However, our two-dimensional analysis methods reveal a predominant step size of ~12nm. By modeling the theoretical steps available to dynein taking into account the microtubule lattice and curvature, as well as the possible distance of the fluorescent probe from the microtubule surface, we hypothesize that dynein does not typically step on the same protofilament. Furthermore, we find that dynein's side steps occur with equal frequency to the left and right, suggesting symmetry in dynein's stepping mechanism. Our results suggest that dynein move on the same face of the microtubule, but with considerably more flexibility than the opposite polarity motor conventional kinesin.

Reck-Peterson, S.L., Yildiz, Y., Carter, A.P., Gennerich, A., Zhang, N., and Vale, R.D. (2006). Single molecule analysis of dynein processivity and stepping behavior. Cell 126, 335-348.

2478-Pos Board B464

Structural Analysis of Cytoplasmic Dynein Tail Domain

Yuta Watanabe, Toshiyuki Kitai, Takuya Kobayashi, Takashi Murayama, Takayuki Takahagi, Yoko Y. Toyoshima.

Cytoplasmic dynein is microtubule-based motor protein utilizing the energy from ATP hydrolysis and plays fundamental roles in various cellular activities including mitosis, vesicle transport and cell migration. Cytoplasmic dynein is a huge protein complex consisting of the head domain and the tail domain. The head domain consists of six AAA modules and a stalk. So far, many studies have investigated about the head domain, because it contains ATP hydrolysis and motor activities. On the other hand, the tail domain consists of N-terminal third of heavy chains (HC), intermediate chains (IC), light intermediate chains (LIC), and light chains (LC). Although the tail domain is implicated in binding of cargos and some of dynein binding proteins which control motor activities of dynein, for example dynactin, the detailed molecular architecture of the tail domain remains to be unveiled. In this study, we observed cytoplasmic dynein molecules with or without polyhistidine tag (His-tag) by transmission electron microscopy (TEM), performed single particle image analysis and obtained the averaged image of the tail domain. Using nickel nitrilotriacetic acid (Ni-NTA) conjugated gold nanoparticles to label His-tag of recombinant proteins, we identified the positions of N-terminus and C-terminus of IC and determined its orientation in the tail domain. Furthermore, we identified the position of C-terminus of LIC relative to IC. Based on our observations, we propose a new model of the architecture of the dynein tail domain which differs from currently advocating model.

2479-Pos Board B465

Functional Dissection of LIS1 and NDEL1 for Understanding the Molecular Mechanism of Cytoplasmic Dynein Regulation

Takayuki Torisawa, Yuta Watanabe, Akiko Nakayama, Kenya Furuta, Masami Yamada, Shinji Hirotsune, Yoko Y. Toyoshima.

LIS1 and NDEL1 are known to be essential for the activity of cytoplasmic dynein in living cells. We previously reported that LIS1 and NDEL1 directly regulated the motility of cytoplasmic dynein in an in vitro motility assay. LIS1 suppressed dynein motility and inhibited the translocation of microtubules (MTs), while NDEL1 dissociated dynein from MTs and restored dynein motility following suppression by LIS1. However, the molecular mechanisms and detailed interactions of dynein, LIS1, and NDEL1 remain unknown. In this study, we dissected regulatory functions of LIS1 and NDEL1 on dynein motility using full length or truncated recombinant fragments of LIS1 or NDEL1. The C-terminal fragment of NDEL1 dissociated dynein from MTs, whereas its N-terminal fragment restored dynein motility following suppression by LIS1, demonstrating that the two functions of NDEL1 localize to different parts of the NDEL1 molecule, and that restoration from LIS1 suppression is caused by the binding of NDEL1 to LIS1, rather than to dynein. The truncated monomeric form of LIS1 had little effect on dynein motility, but an artificial dimer of truncated LIS1 suppressed dynein motility, which was restored by the N-terminal fragment of NDEL1. This suggests that LIS1 dimerization is essential for its regulatory function. These results shed light on the molecular interactions between dynein, LIS1, and NDEL1 and the mechanisms of cytoplasmic dynein regulation. Furthermore, we performed EM study to investigate the binding sites of LIS1 and NDEL1 on dynein molecule and to examine the conformational changes induced by the binding of LIS1 or NDEL1. Our findings will provide the new insights into the molecular mechanism of the regulation of cytoplasmic dynein by LIS1 and NDEL1.

2480-Pos Board B466

Highly Purified Inner-Arm Dynein E from *Chlamydomonas Reinhardtii* Studied with *in vitro* Motility Assay

Youské Shimizu, Hitoshi Sakakibara, Hiroaki Kojima, Kazuhiro Oiwa.

Green alga *Chlamydomonas reinhardtii* has two flagella at the front of the cell and beats them to swim. A flagellium is a biomolecular machine that consists of various proteins in a highly regulated manner. It is considered that an alignment of the various species of dynein proteins, which generate power using energy released upon ATP hydrolysis, plays important roles in the flagellum beating. Recently, great progress has been made in understanding of construction of a *Chlamydomonas* flagellum by the use of cryo-electron microscopic tomography. Dyneins are arranged in 96-nm periodic manner for a direction of a longitudinal axis of a flagellum. 8 species of dyneins are called as ''inner-arm dyneins''. In 96-nm period, they make specific 4 pairs and are arranged in equally-spaced 4 places.

Making use of *in vitro* motility assay, we can evaluate activities of dyneins as ''microtubule sliding''. Dynein ''c'' slides microtubules relatively fast among the 8 inner-arms, and is well-studied. It has shown that dynein c pairs with dynein ''e'' in a flagellium. However, properties of dynein e are not known much. One reason for the difficulty is that anion exchange chromatography, a major purification method for inner arm dyneins, cannot separate dynein e and dynein d well. We obtained highly purified dynein e and c using two steps of anion exchange chromatography. We will present properties of dynein e and of a mixture of dynein e and c.

2481-Pos Board B467

Unbinding Force of Cytoplasmic Dynein

Taketoshi Kambara, Tomohiro Shima, Hideo Higuchi.

Dynein is a molecular motor that moves toward the minus-end of microtubules using the chemical energy of ATP hydrolysis. Cytoplasmic dynein play roles in positioning the Golgi complex and other organelles in cells, movement of chromosomes, and positioning the mitotic spindles during mitosis. Force generation by a dynein molecule, thus, is one of the important factors for understanding molecular properties of dynein. To examine the binding mode between dynein and a microtubule, we measured the unbinding force of dynein in various nucleotide conditions. We used truncated C-terminal motor domain, thus we can eliminate possible effects of tail region and/or accessory proteins on the motor activity of dynein. Dynein with the biotin-tag was attached to avidin-coated polystyrene bead. The bead was trapped by optical tweezers, and the external load was imposed by moving the stage. When the stage was moved at 160nm/sec (loading rate is 5.9 pN/sec), the mean value of the unbinding force of strongly bound state of dynein was 5.7 - 6.6pN upon backward loading. The unbinding force was 20-35% smaller when force was applied to the minus end of microtubules. These data indicate that dynein unbinds from microtubules easily toward the minus end of microtubules to which dynein moves.